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Two Cases of Post Transplant Lymphoproliferative Disorder in Lung Transplant Recipients

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Post-transplant lymphoproliferative disease (PTLD) is a serious, often fatal complication after solid organ transplantation. The incidence of PTLD is greater among heart (2～13%), lung (12%) and heart/lung (5～9%) transplant recipients than among liver (2%), renal (1～3%) and bone marrow (1～2%) transplants recipients. The difference in the incidence of PTLD may be partly attributed to the higher dose of immunosuppressant therapy used for heart and lung transplantation. The Epstein-Barr virus (EBV) infection status of the donor and recipient before a transplant, and high dose of immunosuppressive drugs are considered major risk factors. Recently, 2 cases of PTLD in a single lung and a heart-lung transplantation recipient were encountered. Both patients presented with multiple pulmonary nodules in the transplanted lung, which developed 6 months and 2 years after the transplantation, respectively. Following a transthoracic lung biopsy for diagnostic confirmation, one patient underwent chemotherapy for PTLD and the other conservative care for an accompanying viral infection. Both patients showed rapid clinical deterioration, without response to treatment, and then rapidly succumbed. Herein, our experiences are reported, with a review of the literature.

Key Words : Post transplant lymphoproliferative disease (PTLD), Epstein-Barr virus (EBV)

INTRODUCTION

Post-transplant lymphoproliferative disease (PTLD) is a serious, often fatal complication after solid organ transplantation[1]. The incidence of PTLD is greatest among heart (2～13%), lung (12%), and heart/lung (5～9%) transplant recipients, but occurs less frequently in liver (2%), renal (1～3%), and bone marrow (1～2%) transplant recipients[2-4].

The patient's age, transplanted organ, Epstein-Barr virus (EBV) status of the donor and recipient before transplantation and the dosage of immunosuppressive drugs are considered risk factors[1, 5]. These differences in the incidence of PTLD may be partly attributed to the higher doses of immunosuppressive drugs necessary following heart and lung transplantation[4]. Other possible contributing factors may be bronchus-associated lymphoid tissue (BALT) and nodal tissue harboring EBV in the donor transplant. EBV infection, either preexisting in the recipient or acquired from the donor, is strongly implicated in the pathogenesis of PTLD[4, 6]. Immunosuppression may permit uncontrolled proliferation of EBV-stimulated B cells by inhibition of suppressor T cells, from polyclonal to monomorphic and monoclonal proliferations. Monoclonal proliferations can subsequently accrue mutations of oncogenes or tumor suppressor genes, and lead to gain a fully malignant behavior and loss of responsiveness to immune regulation. Moreover, mutation of *c-myc*, *N-ras* and *p53* genes has recently been implicated in the terminal progression of PTLD[3, 7].

The peak incidence of PTLD occurs 3 to 4 months after transplantation, but may develop as early as 6 days later. Patients with early onset PTLD, within the first year, have a

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better prognosis than those with one developed late onset[1]. In lung transplant recipients, PTLD may develop in the allograft lung, lymph nodes or other MALT sites, which frequently involves extranodal locations, including the lung. A lung involved by PTLD may present as nodular or diffuse reticulonodular infiltrates, as solitary or multiple lung masses, or as hilar and mediastinal lymphadenopathy[8], and pulmonary nodules often have foci of necrosis. Histologically, PTLD can range from polymorphic B cell hyperplasia to monomorphic B-cell proliferation that is indistinguishable from diffuse large cell or immunoblastic lymphomas[3, 5]. These lymphomas are generally high grade with diffuse large cell, immunoblastic, or small noncleaved cell Burkitt or Burkitt-like morphologies.

Herein, our experience of two recently encountered cases of PTLD following a lung transplantation, which demonstrated an aggressive clinical behavior, is reported, with a review of the literature.

CASE REPORT

Case 1.

A 42-year-old man was admitted with a fever and chills for 3 days. His past medical history included dyspnea on exertion, which had developed from childhood, and was diagnosed as having a large patent ductus arteriosus with Eisenmenger syndrome at the age of 20. Six months prior to this presentation, the patient had undergone heart-lung transplantation due to progressive dyspnea, chest pain and peripheral cyanosis. His preoperative examination revealed cytomegalovirus (CMV) IgM

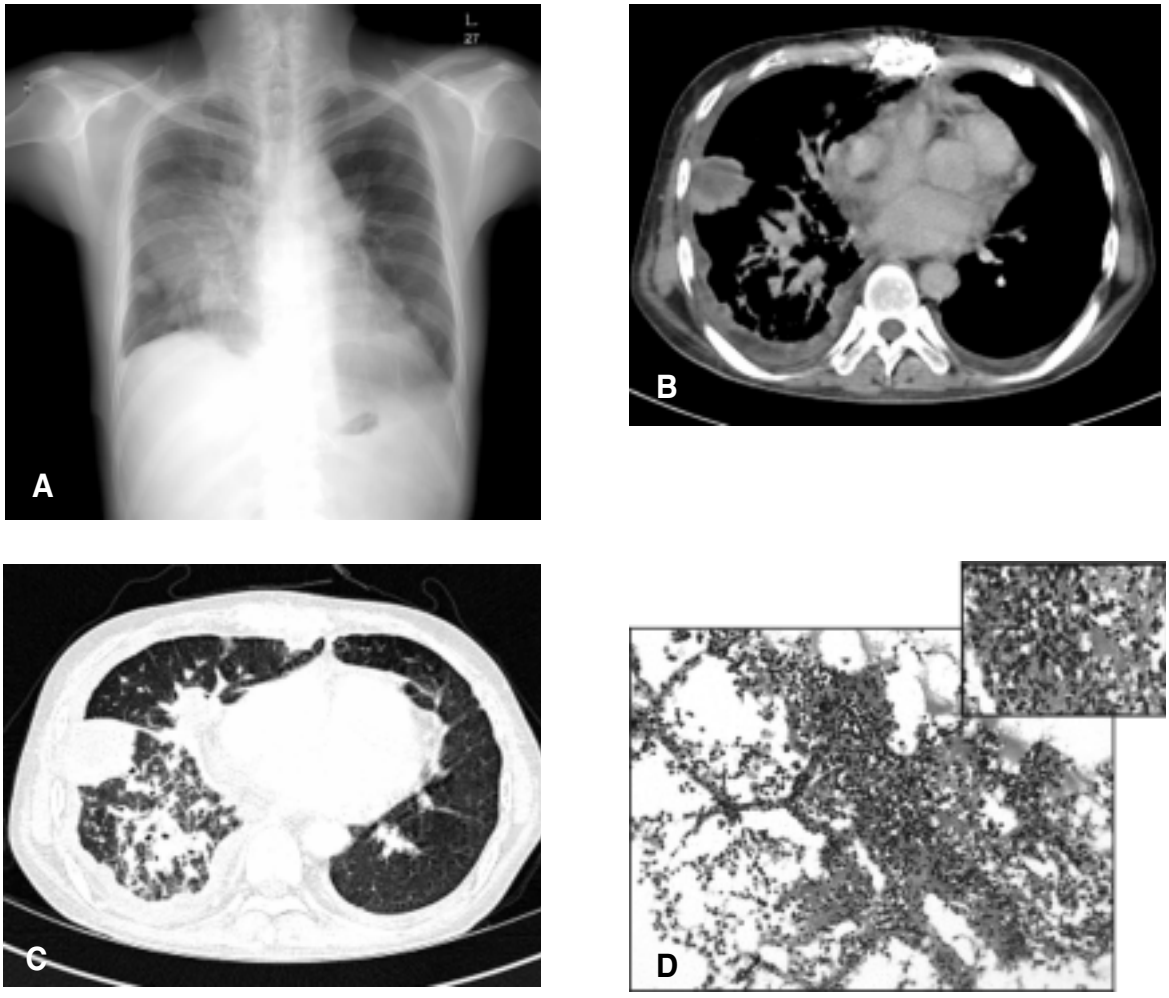

Figure 1. A plain chest radiograph (A) and a computerized tomograph (B, C) of case 1, revealing multiple, variably sized nodular lesions in both upper lung fields. Multiple mediastinal lymphadenopathies are also shown in the right lobe paratracheal and subcarinal areas. Microscopic findings of a transthoracic lung biopsy show irregular thickening of the alveolar septa and perivascular connective tissue, due to the diffuse infiltration of atypical lymphoid cells, which were confirmed as being of B-cell lineage by immunohistochemical staining (D, × 100 & × 200, H-E).

antibody negative, CMV IgG antibody positive, Herpes simplex virus (HSV) IgM antibody negative, HSV IgG antibody positive, Varicella Zoster virus (VZV) IgM antibody negative and VZV IgG antibody positive. The tests for EBV early antigen (EA)-IgM was positive, EA-IgG negative and Epstein-Barr nuclear antigen (EANA) IgG positive. The donor was a 25-year-old male whose preoperative examination revealed CMV IgM antibody negative and CMV IgG antibody positive. The test for EBV EA-IgM was negative and EA-IgG positive. During the immediate postoperative period, the recipient suffered gastric ulcer perforation and underwent a subtotal gastrectomy and gastrojejunostomy. Two months after the transplantation, he developed spiking fever with right lower lung field haziness and an accompanying pleural effusion, which was treated under the impression of bacterial pneumonia. One month later, he suffered a sudden fever and chill, and was admitted for further evaluation.

On physical examination, he had a chronic ill-looking appearance and a pale face. His chest examination revealed coarse breathing sounds in both lung fields and a grade I/IV systolic murmur in the aortic and tricuspid areas. Laboratory tests showed a white cell count of 11,090/μL, hemoglobin 9.2 g/dL, platelet 315,000/μL, Wintrobe erythrocyte sedimentation rate (ESR) 5 mm/hr (reference range; 0-10 mm/hr), serum protein 5.1 g/dL, albumin 2.4 g/dL, aspartate aminotransferase (AST) / alanine aminotransferase (ALT) 22/21 IU/L (reference range; <37/<43 IU/L, respectively), creatinine 0.8 mg/dL, CMV IgM antibody positive, CMV IgG antibody positive, urine CMV-PCR negative, HSV antibody IgM negative, HSV antibody IgG positive, VZV antibody IgM negative and VZV antibody IgG positive. The tests for EBV revealed EA-IgM antibody positive, EA-IgG antibody negative, EBNA IgG antibody positive and EBV-PCR negative. A plain chest radiograph and computerized tomography revealed multiple, variably sized nodular lesions at the apical segments of both upper lungs, anterior segment of the left lung, posterobasal segment of the left lower lobe, hilar portion of the right middle lobe and superior segment of the right lower lobe. Multiple mediastinal lymphadenopathies were also seen in the right paratracheal and subcarinal areas. Minimal pleural effusion was also observed in the right lower thorax. The other findings were unremarkable (Figure 1A-1C). A computed tomogram-guided transthoracic lung biopsy was performed. Irregular thickening of the alveolar septa and the perivascular connective tissue, due to diffuse infiltration of atypical lymphoid cells, were observed. These atypical lymphoid cells were confirmed as B-cell lineage by immunohistochemical staining. The EBV-DNA reaction was positive by *in situ* hybridization, but light chain restriction could not be confirmed (Figure 1D).

Therefore, the patient was diagnosed with PTLD presenting as a polymorphic subtype. His immunosuppressant regimen was adjusted according to the diagnosis. Thirty days after admission, the patient began to complain of visual disturbance, and was treated with intravitreal ganciclovir and foscarnet under the diagnosis of CMV retinitis. The chest lesion continued to progress, with multiple infectious foci, and multiorgan failure developed. He died 42 days after admission.

Case 2.

A 33-year-old woman was admitted for evaluation of multiple air space nodules in the left lung field. Seven years before this presentation, she began to suffer from progressive dyspnea and dry cough. Four years before presentation, she was transferred to a general hospital and diagnosed as having severe emphysema (FVC 1.34 L (35.0%), FEV_1 0.61L/sec (20.0%), DLCO 53% of expected value). She was a healthy Hepatitis B virus (HBV) carrier and her α 1-antitrypsine levels were 189, 381 and 225.3 mg/dL, by three separate tests. Two years before presentation, she underwent left lung transplantation and had been administered cyclosporine A, azathioprine and corticosteroid for immunosuppression, and lamivudine for HBV management. Preoperative serologic tests revealed CMV IgM antibody negative, CMV IgG antibody positive, HSV IgM antibody negative, HSV IgG antibody positive, VZV IgM antibody negative and VZV IgG antibody positive. EA-IgM antibody was positive and EA-IgG antibody negative. There was no information regarding the donor. During 18 months of follow-up after the transplantation, she showed no signs of rejection or an infectious event. However, a routine follow up plain chest radiograph showed multiple air space nodules in the transplanted lung.

On physical examination, her body temperature, pulse and respiration rates were 36.2℃, 74/min and 20/min. She had a chronic ill-looking appearance and a moon face, although the lung sounds were clear in both lung fields without rales. Laboratory tests revealed a white cell count of 5,440/μL, hemoglobin 11.7 g/dL, platelet 232,000/μL, C-reactive protein (CRP) quantitation 25.9 (reference range; 0～8 mg/L), Wintrobe ESR 58 mm/hr, serum protein 5.6 g/dL, albumin 3.2 g/dL, AST / ALT 19/12 IU/L, BUN 33 mg/dL and creatinine 0.9 mg/dL. Urine analysis was normal and a tuberculosis skin test was negative. Serologic tests indicated that she was CMV IgM and IgG antibodies positive. A CMV PCR from bronchial washing fluid was negative. The EA-IgM and IgG antibodies were positive, and the EBNA-IgG antibody was positive, which is a typical reactivity pattern for EBV reactivation, e.g. EBV-associated lymphoproliferative diseases or epithelial carcinomas, such as nasopharyngeal carcinoma. EBV-PCR using whole blood was negative. Chest high resolution computed tomogram revealed multiple air spaced nodules at the left upper lobe and

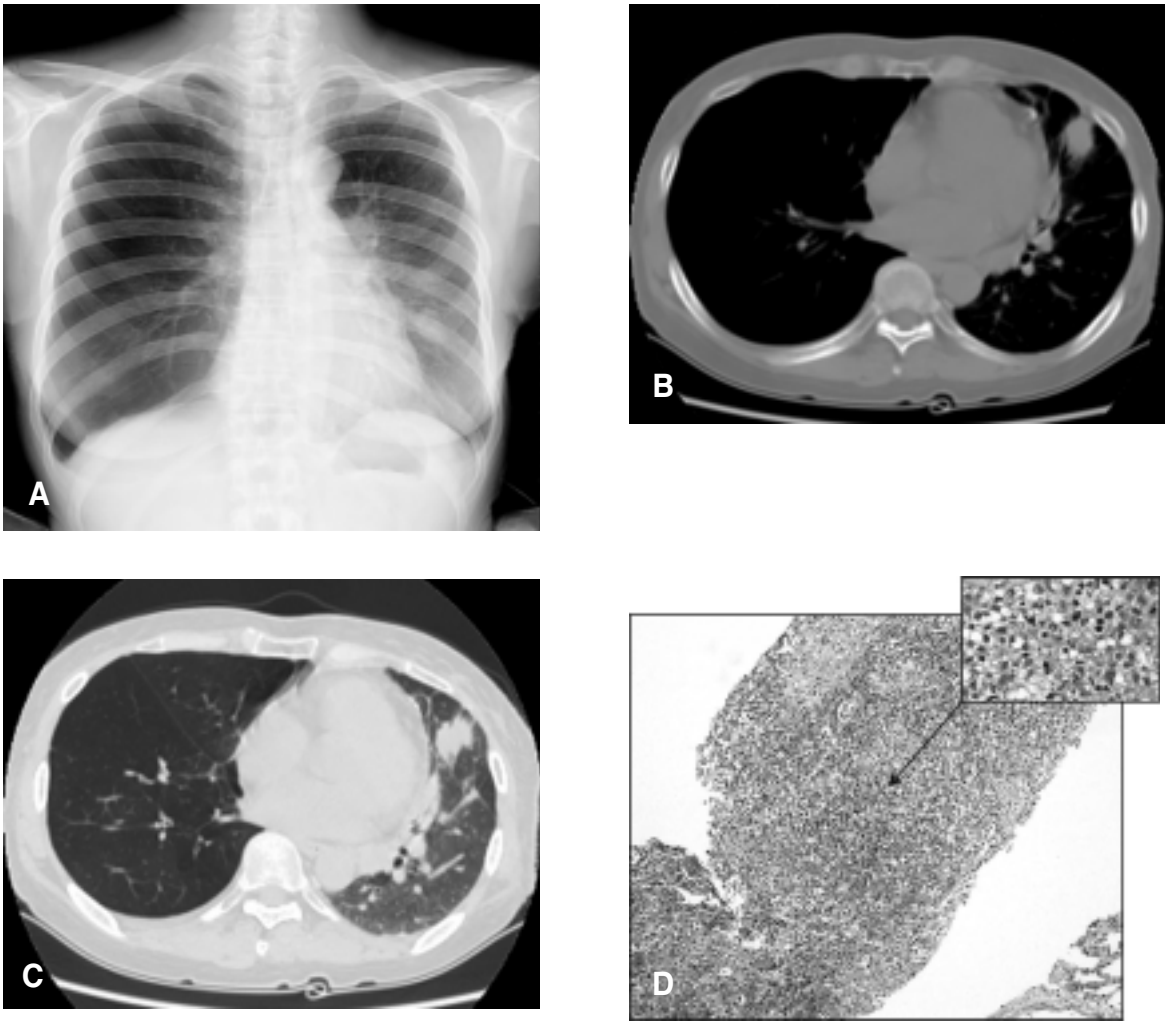

Figure 2. Radiograph of case 2 showing multiple air spaced nodules, which had speculated margins accompanied by peripheral ground glass opacity at the left upper lobe, with collapse of the lingular segment. The native right lung shows severe emphysematous changes (A, B, C). A percutaneous transthoracic needle biopsy specimen shows aggregation of lymphocytes, and an immunohistochemical study of these revealed a positive reaction to L-26, but negative reactions to UCHL-1, Kappa, Lambda, cytokeratin and Ki-1, suggesting a malignant lymphoma, which was later confirmed as a high grade B-cell type malignant lymphoma (D, × 100 & × 200, H-E).

collapse of the lingular segment. These nodules had speculated margins with accompanying peripheral ground glass opacity, suggesting inflammatory changes (Figure 2A-2C). A comparison of inspiratory and expiratory phase images showed neither mosaic perfusion nor small airway disease. The native right lung showed emphysematous changes, with no interval change. A percutaneous transthoracic needle biopsy was performed on a nodule in the left upper lobe. These nodules consisted of lymphocytes, which by immunohistochemical study revealed positive reactions to L-26 and negative reactions to UCHL-1, Kappa, Lambda, cytokeratin and Ki-1 in the tumor cells. A malignant lymphoma, of high-grade large B-cell type, was confirmed (Figure 2D).

She was given a chemotherapeutic regimen comprising of cytoxan 1200 mg, vincristine 2 mg and prednisone 100 mg for 5 days, and one-week later epirubicin 90 mg was administered before being discharged. Two weeks later the patient developed sudden dyspnea and died of pulmonary edema and a pneumothorax.

DISCUSSION

The incidence of PTLD after lung transplantation is 2-fold higher than that seen after any other type of organ transplantation[1]. Primary EBV infection has been implicated as the

major risk factor for PTLD, particularly for EBV-naive patients who seroconverted after the lung transplantation[9]. A 20-fold increase in the risk of developing PTLD was reported to occur in EBV-seronegative vs EBV-seropositive lung recipients after transplantation[10]. In our cases, serologic EBV testing of the recipients showed that EA-IgM was positive and EA-IgG negative, suggesting a recent EBV infection prior to the transplantation.

In the local community, EBV spreads by contact with oral secretion, but has been transmitted by blood transfusion and bone marrow transplantation in a hospital setting through the EBV receptor (CD21), which present on the surface of B cells and epithelial cells. The most likely source of EBV infection through solid organ transplantation is transmission through an organ from an EBV-positive to an EBV-seronegative recipient[11]. The exact mechanism by which the donor virus is transmitted from the transplanted organ to the recipient is unknown, although in solid organ transplant recipients, virus-infected cells in PTLD of recipient origin have been identified[12]. Thus, the donor virus may either be transmitted in a cell-free state or released by donor cells in the recipient. A sufficient number of EBV-carrying B cells may remain in the blood within the allograft and pass into the circulation of the recipient; the virus would then enter the lytic cycle and infect the recipient B-cell population[11]. Alternatively, heart and lung tissue may contain foci of EBV-infected cells, which may act as sites of virus replication. EBV infection in a naive recipient, occurring in the setting of altered host immunity, leads to uncontrolled proliferation and transformation of recipient B cells infected with EBV. These possible patterns of events require identification of high-risk causes and the development of effective therapeutic modalities. A recent study found that continuous, specific anti-viral prophylaxis in high-risk EBV-seronegative recipients significantly reduced the incidence of PTLD after lung transplantation in the absence of induction therapy[13].

Lymphoid proliferations that are morphologically indisting-uishable from a malignant lymphoma occur with greater frequency in organ transplant recipients than in the general population; but are unlikely to progress to malignant lymphomas in immunocompetent patients, but often regress if the immunosuppression is sufficiently reduced[3, 5]. Polymorphic B-cell hyperplasia is associated with a mixture of small lymphocytes, plasma cells and immmunoblasts, without significant cytologic atypia. These cases are usually polyclonal and respond to reduction of immunosuppression. Monomorphic PTLD has sheets of large transformed monoclonal cells or immunoblasts. Despite their overt malignant appearance, some monomorphic tumors regress after reduction of immunosuppression. Non-responders require chemotherapy, although they usually succumb to a progressive disease. Neither the histological appearance nor clonality can accurately predict the response to modulation of immunosuppression. Over the past decades, numerous attempts have been made to classify these lesions using morphology, immunohistochemistry and monoclonality, but none of these tools alone or in combination was found to accurately predict the biological behavior[5, 7]. Monoclonality is considered an adverse prognostic feature. However, some monoclonal PTLDs regress with reduced immunosuppression. Conversely, a subset of polyclonal lesions has a more aggressive course[5]. The first case was confirmed as polymorphic, and was treated by conservative means by adjusting the immunosuppressant and anti-viral agent used to treat the accompanying CMV infection, whereas the second case was suspected as monomorphic PTLD, and treated aggressively using a chemo-therapeutic regimen, but did not respond to treatment.

Because PTLD treatments are most effective in the early stage, prior to the occurrence of progression of monomorphic or monoclonal proliferations[2, 14], not only the early detection of PTLD, but also the identification of the clonality of PTLD, are of importance in planning the means of treatment.

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